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HEAVILY LOADED COLUMNS IN HIGH-SPEED LIQUID CHROMATOGRAPHY

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HEAVILY LOADED COLUMNS IN HIGH-SPEED LIQUID CHROMATOGRAPHY
(Das Verhalten von schwerbeladenen Trennsaelen in der Hochdruck-Fluessigchromatographie)

by

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The stability and the lifetime of heavily loaded columns are determined by the pore structure of the support. If linear velocities up to 5-10cm/sec are wanted the optimum pore diameter should not exceed 500 A. The retentions are influenced by the support if its specific surface area is larger than 10 m^2/g, especially if the samples are capable of hydrogen bonding. This influence can be reduced by chemical modification of the support. Optimum efficiency is achieved by such supports. Heavily loaded column are well suited for semi-preparative separations, since the maximal sample size is around 10 mg per gram of a stationary liquid phase. Speedy separations can be achieved by flow programming.

1. Introduction

The application of heavily loaded columns used in gas- as well as liquid chromatography has been described (1-4). The advantages of such columns are that they can be heavily loaded, have a high peak capacity and high stability against changes in their retention volumes through losses in the stationary phase. The support material for the stationary liquid must fulfill certain

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conditions regarding specific surface area, pore volume and pore diameter (4). In previous investigations silica gels of various manufacture and different physical properties had been used. In order to exclude effects caused by the nature of the support material, Porasil, a uniform starting material was used in the investigations reported here which is commercially available with different physical properties. We could study, therefore, with approximately the same pore volume, the effect of the specific surface area on the retention characteristics. For purposes of comparison one support having a chemically modified surface and one inert support, Chromosrb, were studied side by side. In all cases, however, the pores of the support materials used were filled completely with the developing liquid.

2. Experimental Conditions

Apparatus:

The liquid chromatograph used was shop-made and has been previously described (5).To dampen the pulses and to control the column overhead pressure the previously described regulatory unit was used (6). A modified differential-refractometer (model R 4; Waters Ass., Framingham, Mass., USA) was used to monitor column effluents. (7) The samples were loaded directly onto the top of the columns by means of high pressure syringes (type HP 305; Hamilton, Whittier, California, USA).

All measurements were made at 34°. The reservoir, the preliminary and the separating columns were connected to a liquid-thermostat (type NB, Messgeraete-Werk Lauda, Lauda, B.R.D). All tubings were provided with additional insulation of asbestos. Under these conditions the noise of the detector used was of the order of 1.5×10^{-7} R.I. units.

Separation Columns:

Seamless drawn-out copper tubing of 50 cm x 2 mm i.D. was used as column. Before use the columns were cleaned by rinsing with concentrated HCl, water and aceton. The stationary phase was packed dry, in sections and under light vacuum vibrating and pushing up the columns during the operation.

Reagents:

n-Heptane was used exclusively as the mobile phase. The stationary phase was 3,3'-oxydipropionitrile (ODPN) with a density of 1.06 g/ccm (Merck, Darmstadt, B.R.D.). The mobile phase was saturated with ODPN at 34.2°. To minimize the washing out of the stationary phase a preliminary column (10 cm x 4 mm i.D.) filled with silica gel (coated with 1.1 g ODPN/g silica gel) was used.

The physical properties of the support materials used are summarized in table 1. All Porasils (Waters Ass.) were freed of organic contaminants by boiling them for two hours in concentrated sulfuric acid containing about 10 % nitric acid, before use. After washing with water until neutrality had been reached, and drying at 200°, the Porasils were coated with the stationary phase. The work of others (4) has shown that nearly the entire pore volumn can be filled with stationary phase before the particles become sticky. With the Porasils used in this study the amounts of ODPN cited in the literature (8) could not be triturated. Therefore, of all dried preparations the weight poured was determined, which is approximately inversely proportional to the pore volume. For this purpose an accurately weighed amount (at least 5 ml) were packed into a graduated cylinder (10 ml) with tapping and pushing up until no more change in the packed volumn could be observed. The determination of the poured weight was used to control the packing of the separation column as well as to enable us to control the coating of the support with an error

of less than 3%. The poured weight that was determined for the uncoated support showed better agreement with the maximal achievable coating that values for the pore volume taken from the literature.

The coating of the support with the stationary phase was done as usual, by dissolving the ODPN in methylene chloride and slow evaporating of the solvent after addition of the support in the rotary evaporator. Only enough separation liquid was applied such that the stationary phase remained just dry and fluid.

For purposes of comparison a chemically modified support material was included in the study. The support material (Porasil C) was for this purpose esterified in the usual way with 3-hydroxypropionitrile (11) (Porasil C-OPN). This modified support material is referred to below as "brush". The brush could be further coated with 0.72 g ODPN/g brush.

3. Results and Discussion.

Relative Spread of the Bands:

The dependency of the band spreading on the flow rate was studied for all support materials in the range of 0 - 8 cm/sec. Fig. 1 shows a typical curve of h = f(u). For all columns and samples studied the curve is described by the empirical function

(1) $$h = A + C.u$$

within the given range of flow rates. The constants A and C, together with the k' values for the systems studied are summarized in table 2. For reasons that will be discussed more extensively later, the values obtained with Porasil F as support are not contained in the table. The values of the constants A and A were determined from a linear regression function and have a standard deviation of ± 10% for the C-terms and ± 15% for the A-terms. The C-terms exhibit the well-known dependency on the k'-value that must be expected for heavily

loaded columns. Maximal C-terms are reached with k'-values of 1, as predicted by theory (12). At high k'-values (above 30) the C-terms are comparable to those of the inert peak. The lowest C-terms of all systems studied were obtained with the chemically modified support (Porasil C-OPN). The reason for this may be the surface that is esterified with nitrile-groups. For supports with large pore diameters (Porasil E and F) relatively poor C-terms were measured. These particles tend to stick together and can, therefore, not be as evenly packed. The A-values lie between 1-2mm and are, thereby, relatively high. This means that the h-values, especially for the low C-terms, are still affected by the A-terms, even at high linear flow rates. For an A-term of 2 mm and a C-term of 100 msec one obtaines a share of the A-term on the h-value of 67% at a flow rate of 1 cm/sec. Only at flow rates of about 10 cm/sec does the percent share of the A-term in the band spread fall below 20%. The picture is worse yet if one considers the lowest C-term, namely 30 msec. Here the A-term contributes between 87% (for 1 cm/sec) and 40% (for 10 cm/sec) to the band spreading. Since A is dependent on d_p, better, i.e. lower h'values should be achieved by decreasing the particle diameter. Experiments with particle diameters of 10 micrometers are presently being performed.

Determination of the Film Thickness:

In the values of the C-terms in table 2 the mass transport in the mobile phase (C_m) as well as in the stationary phase (C_s) is included. The strong dependency of the C-terms on the k'-values with a maximum at k'-values of 1, found for heavily loaded separation columns, suggests that the C_s-term plays here a non-negligible role. According to the theory of chromatography (13) the contribution to band spreading, caused by diffusion of the sample in the stationary phase can be described by

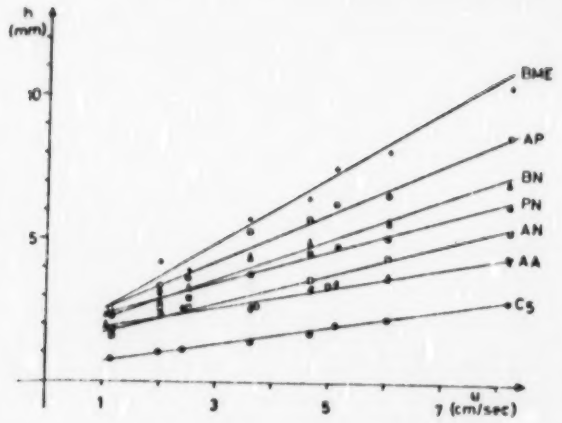

Fig. 1. Typical h=f(u) curve. Stationary phase, 0.72 g ODPN/g Porasil C-OPN "brush"; column: 50 cm x 2 mm i.D.; mobile phase: n-heptane. C_5=n-pentane(k'=0); BME=benzoyl methylester(1.3); AP= acetophenone (3.6); BN=benonitrile (5.5); PN=propionitrile (12.6); AN=acetonitrile (31.7); AA=anisic alcohol (61).

$$(2) \qquad C_s = q \cdot \frac{k'}{(1+k')^2} \frac{d_f^2}{D_s}$$

where D_s is the diffusion coefficient of the sample in the stationary phase; d_f is the film thickness of the stationary phase and q is a factor that depends on the geometry of the pore. For cylindrical pores q = 2/3, whereas for small pores q = 0.25. To a first approximation the diffusion coefficient is the same for all samples analyzed (calculated according to Wilke and Chang to be 1.27 x 10^{-6} cm^2/sec (14)); nor it is to be expected that d_f is dependent on the type of sample. If these assumptions are valid, the C_s-term should exhibit a linear dependency on the k' function in equation (2). The C-term for various columns is plotted against this function in fig. 2. The slope of the straight line thus obtained gives, when multiplied by q, the time the sample remains in the stationary phase. The film thickness, or pore depth, so calculated is in all cases about 15 micrometers. This confirms the gaschromatographic measrements (3), which say that the film thickness of the stationary phase is identical with the pore depth for heavily loaded columns, and is of the same order of magnitude as the particle radius.

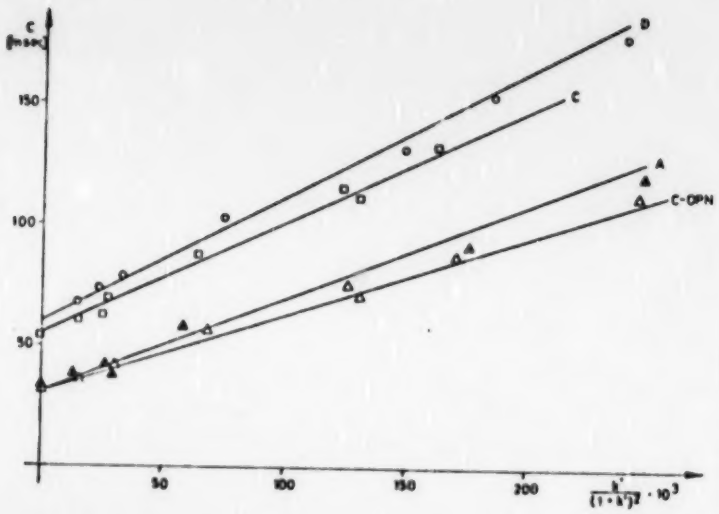

Fig. 2. Dependency of the C-terms on the k'-function in equation (2). A=Porasil A coated with 0.91 g ODPN/g; C=Porasil C coated with 0.79 g ODPN/g; C-OPN=Porasil C-OPN "brush" coated with 0.72 g ODPN/g; D=Porasil D coated with 0.45 g ODPN/g.

Speed of Analysis:

Because of the usual high k'-values for heavily loaded columns the theoretical and actually achieved separation gradations are very similar (4). In fig. 3 the number of effective plates per second is plotted against the linear flow rate. For k'-values between 1 and about 15, between 0.6 and 1 effective plate is produced per second, despite the fact that the particle diameter of 50-56 micrometer is relatively large for high pressure liquid chromatography. The optimal flow rate obtained from the h/u=f(u) curve is for this column between 4-5 seconds. The achievable flow rate with heavily loaded columns is clearly acceptable, even if it is not comparable to that achieved with thin layer particles or columns with small particles.

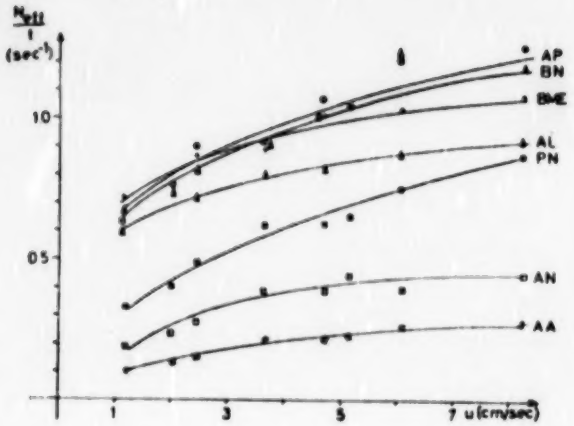

Fig. 3. Effective plates per second vs. flow rate. Conditions as in fig. 1. AL=anisole (k' = 0.9).

Stability of the Separation Columns:

The pore diameter determines the stability of the column. With small pores the stationary phase is better retained by capillary action than with larger ones. When saturated mobile phases are used, the stationary phase is only worn out by mechanical erosion. The smaller the pores, the lesser should be the mechanical erosion, i.e. this erosion should only become noticeable with very high flow rates. A sensitive index for the stability of the columns is the constancy of the k'-values which can be determined to an accuracy of ± 4% without great effort. The flow rate was determined for all phases tested for which the k'-values changed beyond the accuracy given. It turned out that when the critical flow rate is exceeded, the k'-values decrease very rapidly by 40-80%. In addition the noise of the detector increased considerably. In table 3 the flow rates, at which the columns were just stable, are compared with the pore diameters. When Porasil F was used, the k'-values changed noticeably even at flow rates below 2 cm/sec. Therefore values obtained with Porasil F were not recorded in table 2. In order to achieve flow rates that are optimal for heavily loaded columns of about 5 cm/sec without shortening the lifetime of the column, the pore diameter should not exceed 500 A.

Table II: Comparison of the Constants A and C on Different Supports (with maximal coating).

Probe	Porasil A (0.91 g ODPN/g Porasil A)			Porasil C (0.79 g ODPN/g Porasil C)			Porasil C-OPN (0.72 g ODPN/g Porasil C-OPN)			Porasil D (0.45 g ODPN/g Porasil D)			Porasil E (0.34 g ODPN/g Porasil E)		
	k'	A (mm)	C (msec)	k'	A (mm)	C (msec)	k'	A (mm)	C (msec)	k'	A (mm)	C (msec)	k'	A (mm)	C (msec)
n-Pentan	0	1.0	32	0	0.9	52	0	0.4	31	0	1.6	40	0	0.8	56
Toluol	0.3	1.4	76	0.2	1.2	82	0.3	1.1	58	0.2	2.9	81	0.1	1.6	68
Anisol	1.1	1.6	118	0.85	1.6	110	0.9	1.3	106	0.8	4.5	127	0.5	1.7	135
Benzoesäuremethylester	1.7	1.7	143	1.5	1.8	127	1.3	1.4	113	1.2	4.5	179	0.7	1.9	130
Acetophenon	4.5	1.3	130	3.9	1.2	133	3.6	1.7	87	3.1	4.1	153	1.9	1.6	169
Aceton	–	–	–	5.5	1.1	112	5.7	1.6	77	4.5	3.5	142	2.7	1.7	105
Benzonitril	8.2	2.1	100	5.9	1.0	119	5.5	1.4	70	5.3	2.9	140	3.1	1.9	142
Propionitril	15.1	2.1	58	13.3	1.3	89	12.6	1.8	56	11.3	4.9	102	6.9	1.6	76
Phenylpropanol	19.4	1.7	79	16.7	2.2	66	18.7	1.9	61	12.5	4.2	120	8.0	1.5	130
Benzylalkohol	35.8	1.9	42	32.1	1.6	71	31.0	1.6	41	27.8	4.3	79	17.1	1.9	109
Acetonitril	36.7	1.9	39	33.9	1.8	61	31.7	1.2	51	27.9	3.3	74	17.3	1.2	70
Anisalkohol	75.3	1.9	38	64.4	1.5	60	61.1	1.5	36	59.3	4.0	69	36.8	3.2	70

Table III: Maximal Flow Rates for Heavily Loaded Separation Columns

a) Trägermaterial	b) Durchschnittlicher Porendurchmesser (Å)	c) Maximale Geschwindigkeit (cm/sec)
Porasil A	< 100	ca. 15
Porasil C	~ 300	ca. 12
Porasil C-OPN	~ 300	ca. 12
Porasil D	~ 600	ca. 10
Porasil E	~ 1000	ca. 5
Porasil F	> 2000	< 2

a) support material;

b) average pore diameter;

c) maximal flow rate;

This value is in good agreement with the results of others (4) who had found a pore diameter of 100-200A optimal for heavily loaded separation columns.

Effect of the Specific Surface Area on the Retention:

From the k'-values the "chromatographic" distribution coefficients can be calculated. If this distribution coefficient is plotted against the reciprocal of the volume of the stationary phase in the column, the effect of various retention mechanisms can be discerned (15, 16). The Porasils used in this study differ from one another only in the size of the specific sur-

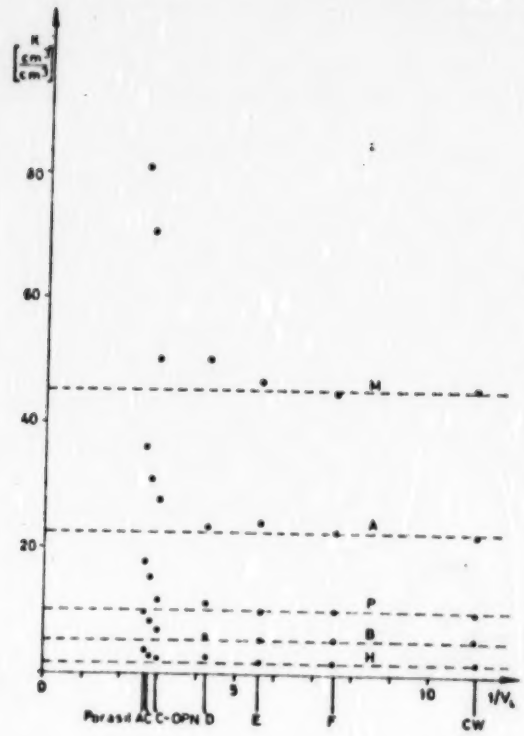

Fig. 4. The dependency of the chromatographic distribution coefficient on $1/V_L^\circ$ for alcohols. H=n-hexanol (K=1.6); B=n-butanol (3.2); P=n-propanol (10.0); A=ethanol (22.4); M=methanol (45.2); The stationary phases are tabulated in tables I and II. The points between two shaded lines belong, in each case, to the lower line.

face area, having nearly the same pore volumes. In fig. 4 the calculated distribution coefficients of aliphatic alcohols are plotted against $1/V_L^\circ$. Only for surface areas below 10 m^2/g (Porasil E) the effect of the surface on the retention, especially of polar alcohols, can be neglected. In these cases the distribution coefficient on a silica gel is identical with that on silanized Chromosorb that is generally considered an inert support. Compounds with low proton activity, such as long-chain alcohols or nitriles, whose behavior is depicted in fig. 5, are hardly affected by the specific surface in their retention properties.

Esterification of the surface of the support with alcohols (Porasil C-OPN) leads to a considerable decrease in adsorptivity of the support material. The distribution coefficient for methanol decreases from about 70 on

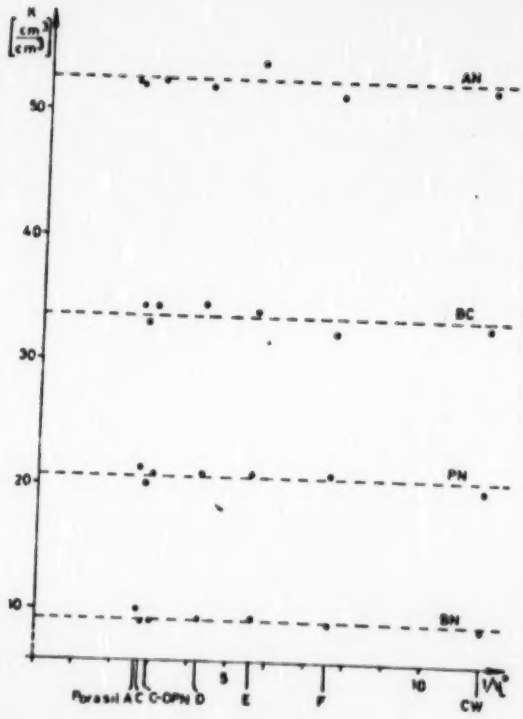

Fig. 5. Dependency of the chromatographic distribution coefficient on $1/V_L^o$ for nitriles. BN=benzoylnitrile (K=9.3); PN =propionitrile (20.7); BC= benzylcianide (33.6); AN=acetonitrile (52.7); Other conditions as for fig.4.

Porasil C to about 50 on "brushed" Porasil A. For the same compound a distribution coefficient of 45 was determined on Chromosorb W. Esterification leads to a decrease of "active" surface and therefore to distribution coefficients similar to those measured on "inert" supports.

The distribution coefficients represented by broken lines in fig. 4, which were measured with Porasil E and F as well as with silanized Chromosorb W, appear actually to be "pure" distribution coefficients, since a plot of the logarithm of the distribution coefficient against the number of carbons yields the expected linearity (cf. fig. 6).

Load Capacity:

The load capacity of chromatographic systems is defined by Snyder (17) as the maximum amount of sample whose retention volume lies no more than

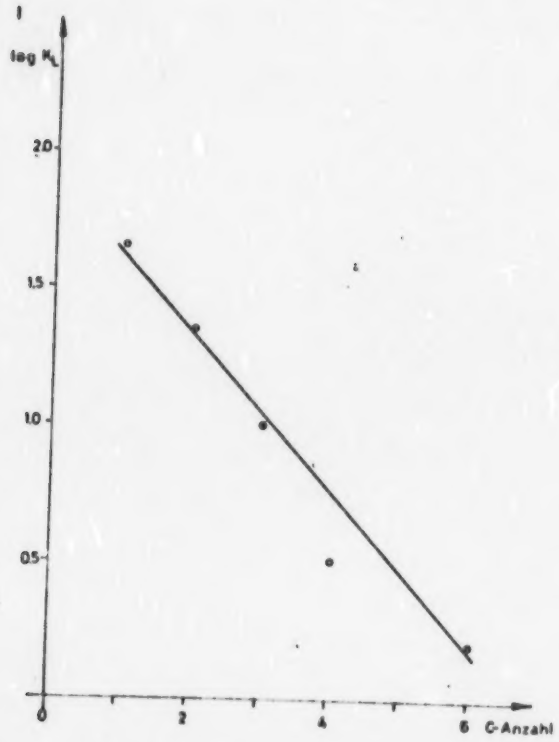

Fig. 6. Dependency of the distribution coefficient on the number of carbons. cf. fig. 4.

10% below that obtained with very small samples and which lies within the linear range of the isotherms. In our experience the increased band spreading indicates more clearly an overload on the separation system than the decrease in retention time, i.e. of the k'-values. In fig. 7 load capacity curves are given for a sample on three different saturated supports. For purposes of comparison the increase in band spreading (left ordinate) and the decrease in k'-values (right ordinate) are plotted as a function of the sample size per gram separation liquid in the column. For all experiments the same column, with a void volume of 1.57 ml, was used. The load capacity of heavily loaded columns lies between 2×10^{-3} g/g separation liquid for Porasil A and about 8×10^{-3} g/g for Porasil C and E. If the change in k'-values is used, a slightly higher load capacity is obtained, namely, about 10^{-2} g/g

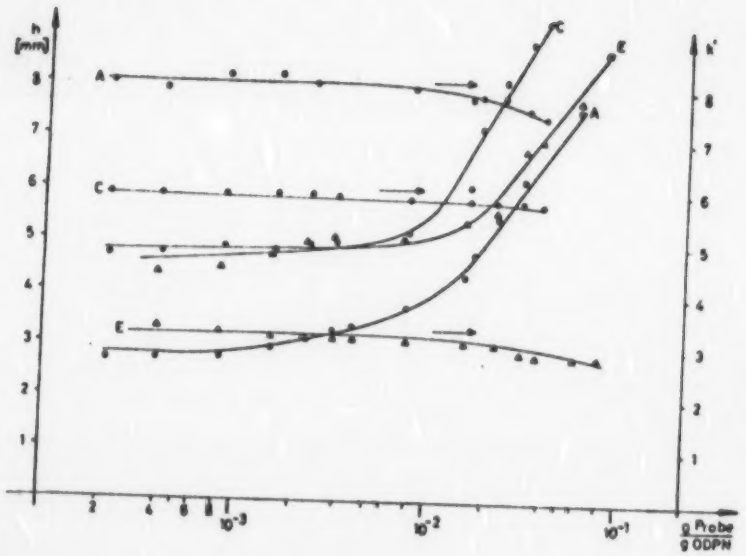

Fig. 7. Load capacity of various stationary phases for benzonitrile. Mobile phase, n-heptane; flow rate 1.97 cm/sec.; temperature 34°. A=0.91 g ODPN/g Porasil A; C=0.79 g ODPN/g Porasil C; E=0.34 g ODPN/g Porasil E.

for all three support materials.

In fig. 8 the load capacity of a stationary phase (0.91 g ODPN/g Porasil A) for samples with different k'-values is plotted. If the change in band spreading is used, a load capacity of about 5×10^{-3} to 1×10^{-2} g/g separation liquid is found, independent of the k'-value. If, on the other hand, the change in k'-values is considered, a somewhat larger load capacity is found which does depend on the k'-value.

Therefore, about 10 mg sample could be loaded onto the column used of 50 cm x 2 mm i.D. without additional band spreading. With samples of such size other analytic procedures and assay reactions can be carried out after separation on the column has been achieved.

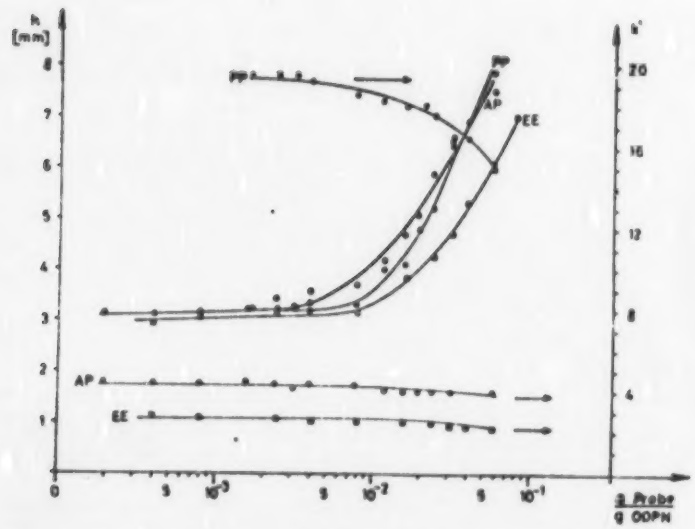

Fig. 8. Load capacity determined with different samples. Stationary phase 0.91 g ODPN/g Porasil A; mobile phase n-heptane; flow rate 2.0 cm/sec.; temperature 34°. EE= emthylacetate (k'=2.8); AP=acetophenone (4.4); PP=phenylpropanol (19.5).

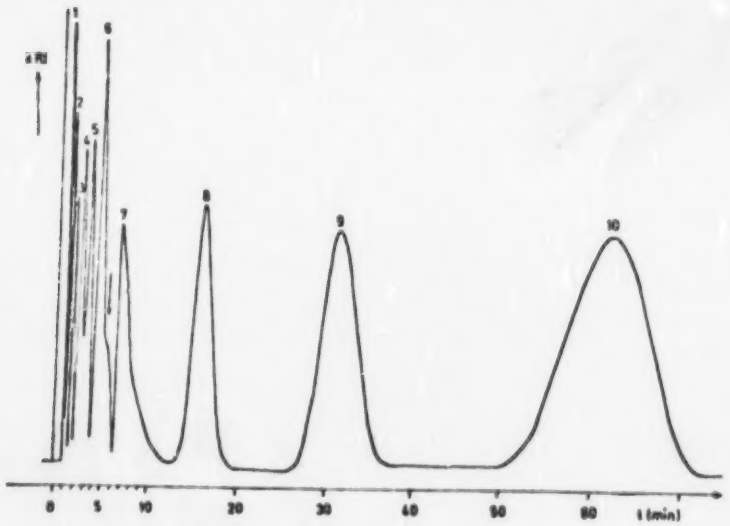

Fig. 9. Separation of a random mixture. column 50 cm x 2 mm i.D.; stationary phase 0.72 g ODPN/g Porasil C-OPN (d_p=50-56 micrometer); mobile phase n-heptane, flow rate 0.88 cm/sec.; Δp=5.5 atm.; temp. 34°;1=n-none(k'=0); 2=thionaphthene (0.3); 3=N,N'-dimethyl aniline (0.8); 4=naphthoquinoline (1.6); 5=quinaldine

(legend to fig. 9 continued): 6=p-benzoyldiazine (3.95); 7= isoquinoline (6.5); 8=phenylpropanol (16.8); 9=benzyl alcohol (32); 10=anis alcohol (63).

Application:

In addition to the micro-preparative application of heavily loaded separation columns already outlined, an example of the possibility of a pressure programming is to be demonstrated. This is possible with such a separation system since the separation columns are, as has been shown, very stable even at high linear flow rates (about 15 cm/sec.). Fig. 9 shows the separation of a random mixture of ten components on Porasil C-OPN, coated with 0.72 g ODPN/g support. Due to the high k'-values and the slow flow rate (0.9 cm/sec) the separation is complete only after more than 60 minutes. But the bands of the samples that are eluted late are still perfectly symmetrical. In fig. 10 the same separation is represented, but in this case the pressure on the column was continually raised from an initial value of 7.5 atm. to 135 atm.. The flow rate increased, as a consequence, from about 1 cm/sec. to 21 cm/sec.. Therefore, the separation was completed in 7 minutes. Even at this extremely high flow rate the column was stable and the base line of the differential refractometer changed only insignificantly. At a constant noise level of 1.5×10^{-7} R.I. units, the base line rose during the pressure change only by 2×10^{-7} R.I. units. Programming the column pressure is well suited to reduce the often relatively long separation times on heavily loaded columns.

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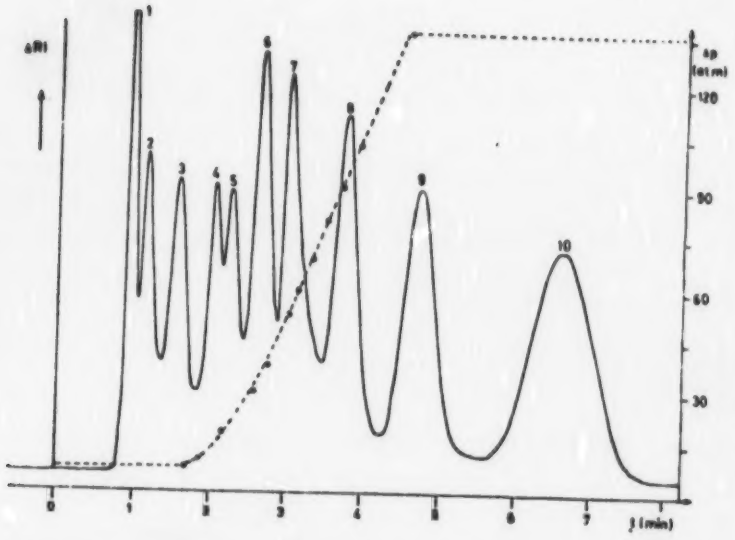

Fig. 10. Same separation as in fig. 9. Same condition, except with pressure program from Δp 7.5 atm. to Δp=135 atm.

4. Summary

The pore structure of solid supports has, for heavily loaded separation columns a great effect on the stability and the lifetime of the columns. To enable one to work with high flow rates (5-10 cm/sec.) the pore diameter should not exceed 500 A. A specific surface area greater than about 10 m^2/g influences the retention characteristic, especially for compounds capable of forming hydrogen bonding. By chemically mofying the surface of the support, the influence of this adsorptivity can be significantly diminished. Thusly modified supports gave columns with the lowest A-and C-terms. The load capacity of such columns is very great (2×10^{-3} to 1×10^{-2} g sample /g separation liquid), such they are well suited for micro-preparative separations. The often long analysis times can be significantly shortened through a programmed pressure increase.

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